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8. Biotransformation Of Priority Pollutants Using Biofilms And Vascular Plants

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Abstract. Microbial filters with and without reeds (*Phragmites communis*) were used to degrade aromatic and aliphatic organics on the EPA priority pollutant list. The initial concentrations of the organics were in the range of 400-1000 $\mu g/\ell$. The contaminated river water was treated under batch conditions. The river water contained sufficient dissolved oxygen to sustain aerobic conditions. The plant-free microbial filter effected the removal of 61-99% of the aromatic compounds in 24 hrs and 39-81% of the aliphatics. The reed/microbial filter system improved the removal rates to 81->99% for the aromatics and 49-93% for the aliphatics in 24 hrs.

Additional Index Words. microbial filter, reed, *Phragmites communis*, priority pollutants.

Introduction. Many studies have demonstrated the ability of microorganisms under defined conditions to degrade aromatic hydrocarbons (Eaton & Ribbons, 1982; Gibson, 1971 and 1977; Keyser *et al.*, 1976; Tabak *et al.*, 1981). Degradation of halogenated aliphatics seems to be more easily accomplished under anaerobic or anoxic conditions (Bouwer and McCarty, 1983 A & B; Bouwer *et al.*, 1981). Recently Wilson and Wilson (1985) found evidence for the aerobic degradation of trichloroethylene. Harber et al. (1983) found that methylotrophic bacteria are capable of oxidizing many halogenated methanes as well as some aromatic hydrocarbon derivatives.

In most of the above studies, microorganisms, especially of the *Pseudomonas* genus, were either isolated from a situation which had been contaminated for some time with toxic organics of interest or were gradually adapted to utilize the organics as a partial or total source of carbon. Microorganisms can adapt to utilize new carbon sources by a process of recruiting

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various genes from existing plasmids to make new plasmids which code for enzymes necessary to convert the carbon sources into compounds useful for energy and cell mass synthesis (Kellogg *et al.*, 1981). Biological processing techniques have been developed which enable adapted microorganisms to be retained in the biological treatment unit for periods much greater than the hydraulic retention time. Young and McCarty (1969) were the first to demonstrate the effectiveness of anaerobic filters or biofilms for domestic wastewater treatment. Further technology in this area has been expanded by Rittman and McCarty (1980), Wolverton (1982), and Wolverton *et al.* (1983). The microbial filters developed by Wolverton (1982) also supported growth of the common reed (*Phragmites communis*) which enhanced the overall removal rate of the filter. Wolverton and McDonald (1981) demonstrated that a reed/microbial filter system could remove 93% of the phenol in a 100 mg/ℓ solution in 24 hours.

To further evaluate the utility of microbial filters, a microbial filter was used to biotransform organics cited on the U. S. EPA priority pollutant list (Government Institutes, Inc., 1985). In addition, an identical microbial filter system supporting growth of the common reed (*Phragmites communis*) was studied simultaneously to assess the additional benefit of using vascular aquatic plants to remove organics and/or their metabolic breakdown products from microbial action on the priority pollutants.

Material and Methods. Experimental Systems. A fiberglas tank was used to mix the organics with fresh river water drawn from the East Pearl River at the National Space Technology Laboratories, Mississippi. To contaminate the river water, a concentrated mixture of organics was prepared in methanol which was added to the river water. The contaminated river water was pumped from the tank into the two microbial filters in a batch-type operation. Consequently, the initial concentrations of organics introduced into both filters were the same.

The microbial filters were troughs, 50.5 cm W x 30.5 cm D x 298 cm L, made of galvanized steel and filled to a depth of 16 cm with rocks (2.5-7.5 cm in diameter) with a top layer of pea gravel (0.25-1.3 cm in diameter) 5 cm deep. Each filter was approximately 2 years old and had been used in other evaluations with domestic wastewater. Therefore, the filters already contained an active biofilm. The reeds were well established with their root systems extensively enmeshed within the filter.

Immediately preceeding each new run, the troughs were drained, and new contaminated river water was introduced. Samples were collected via a bottom valve. Samples were removed initially from the mixing tank, and later at 4 and 24 hr exposure intervals. Data was collected for a total of 48 runs.

Experimental Procedures. Background data on the initial and 24 hr samples was obtained by analyzing for pH, total suspended solids (TSS), total dissolved solids (TDS), total phosphorus (TP), total kjeldahl nitrogen (TKN), total organic carbon (TOC), and chemical oxygen demand (COD) (American Public Health Association, 1981).

The purgeable organics were analyzed with automated purge and trap equipment and gas chromatograph using an FID and 6 ft x 1/4 in glass column packed with 60/80 Carbopak B, 1% SP-1000. The base/neutral extractable organics were analyzed according to Longbottom and Lichtenberg (1982) using a methylene chloride extraction and concentration procedure and gas chromatograph equipped with an FID and a 6 ft x 1/4 in glass column packed with 100/120 Supelcoport, 3% SP-2250.

Results and Discussion. The filters used in this study were two years old and had been used in other projects involved with the stabilization of domestic wastewater by this process. Therefore, the filter surfaces were well covered with an active biofilm. The existing microflora were acclimated for this study by adding the organic concentrate in river water to the filters five days a week for one month prior to data collection.

Background data on the river water over the experimental period is shown in Table 1. The COD is mainly due to the methanol used to make up the organic concentrate mixture. Sufficient concentrate was added to the river water prior to mixing to achieve a 1000 $\mu g/\ell$ concentration of each organic. However, some was lost during the mixing process, and consequently the initial samples obtained just prior to introducing the water into the troughs reflect lower concentrations.

Table 1. Background data on initial and final (24 hr) samples from the two filter systems.

Parameter	Concentration, mg/ℓ		
	Initial	Final	
		Without reed	With reed
pH (Std units)	7.1	7.1	6.9
DO	7.2	2.5	2.4
Temp, °C[1]	24.2	21.7	21.8
TOC	110.6	47.0	41.6
TSS	6.9	7.0	4.6
TDS	103.8	117.2	112.1
TP	1.1	1.4	1.1
TKN	2.8	3.2	2.7
COD	747.4	334.1	317.0

[1]water temp.

The spiked river water contained an average initial dissolved oxygen (DO) concentration of 7.2 mg/ℓ. The systems were still aerobic after 24 hrs. However, biotransformation of the added organics was slow after the first 4 hrs of exposure.

Data for all the organics can be found in Tables 2 & 3. Loss due to volatilization appears to have been relatively minor when analyzing the data for the most volatile organics (Henry law constant, H>3500 torr M^{-1}) such as benzene, ethylbenzene, and chloroform. For example, without reeds the initial benzene concentration was reduced 58% from 721.4 to 302.0 μg/ℓ in the first 4 hrs and only by another 16% from 302.0 to 254.8 μg/ℓ over the 20 hr period between the 4 and 24 hr samples, respectively. Ethylbenzene showed a similar pattern with 62% removal in the first 4 hrs and 11% in the next 20 hrs. Chloroform exhibited a 47% reduction in the first 4 hrs, and over the next 20 hr treatment period an additional 20% reduction was seen. A sterile control to obtain absolute control results of volatilization was impossible to devise considering the size and environment of the filters.

The data in Table 2 shows that biotransformation of the organics was most rapid in the first 4 hrs. The initial rapid kinetics correspond to the period of highest DO concentrations. Aerobic metabolism of chlorinated aromatics such as chlorobenzene has been shown to require as little as 20 min for 95% removal of initial concentrations of 10 μg/ℓ when the DO is maintained at 5 mg/ℓ (Bouwer and McCarty, 1981). In the study by Bouwer and McCarty (1981), the chlorinated aromatics were utilized as secondary substrates.

The microbial filter system without reeds effected the removal of 61-99% of the aromatic hydrocarbons in 24 hrs and 39-81% of the chlorinated aliphatics (Table 2). The reed/microbial filter system improved the removal rates to 81->99% for the aromatics and 49-93% for the aliphatics (Table 3). Complete mineralization of the organics cannot be deduced from the data because only the concentrations of the parent compounds were monitored.

The growth of reeds within the filter beds made a noticeable difference in the data. Two possible explanations are that the reeds directly absorbed a fraction of the organics or that the reeds absorbed organics that were intermediates in the microbial degradative pathways and caused a shift in equilibrium towards product formation.

Both filter systems demonstrated significant removals of the halogenated aliphatic hydrocarbons. Bouwer and McCarty (1981 and 1983*a*) found no aerobic degradation of chloroform, 1,1,1,-trichoroethane, and tetrachloroethylene. However, in the review by Harber *et al.* (1983), several aerobic microorganisms have been identified that can utilize halogenated 1-carbon compounds. Research by Wilson and Wilson (1985) demonstrated that trichloroethylene could be aerobically degraded.

The upper limit concentrations of the 24 hr effluent samples at the 95% confidence level (P=0.95) are shown in Table 4. The average statistical upper limit of discharge for the reed/microbial filter was 36% and 28% lower for aromatic and aliphatic hydrocarbons, respectively, than for the plant-free microbial filter.

Table 2. Average organic concentrations as a function of time in the microbial filter system without reeds.

Organic	Concentration, $\mu g/\ell$						Removal, %
	Initial	(σ)	4 hr	(σ)	24 hr	(σ)	
Aromatic:							
Benzene	721.4	(223.6)	302.0	(103.7)	254.8	(101.5)	65
Biphenyl	821.0	(289.2)	86.9	(34.3)	71.5	(33.4)	91
Chlorobenzene	531.2	(191.1)	244.3	(107.8)	207.9	(88.9)	61
Dimethylphthalate	1032.6	(225.5)	511.2	(122.5)	354.8	(122.8)	66
Ethylbenzene	430.3	(196.5)	163.5	(84.3)	145.8	(68.7)	66
Naphthalene	706.7	(197.2)	100.8	(28.3)	98.2	(30.3)	86
p-Nitrotoluene	980.5	(316.3)	97.1	(59.3)	5.2	(8.2)	99
Toluene	581.4	(210.7)	201.1	(86.3)	171.4	(71.1)	71
p-Xylene	398.1	(186.2)	153.9	(79.3)	129.1	(56.8)	68

Table 2 (cont'd)

Organic	Concentration, μg/ℓ Initial	(σ)	4 hr	(σ)	24 hr	(σ)	Removal, %
Aliphatic:							
Bromoform	640.8	(246.4)	226.4	(149.9)	122.6	(134.0)	81
Chloroform	837.7	(201.3)	441.9	(118.5)	352.3	(166.5)	58
1,2-Dichloroethane	821.6	(166.3)	583.1	(122.9)	498.4	(173.5)	39
Tetrachloroethylene	457.3	(189.1)	280.2	(103.9)	214.8	(92.0)	53
1,1,1,-Trichloroethane	756.1	(391.9)	400.7	(131.8)	325.7	(140.6)	57

σ = standard deviation

Table 3. Average organic concentrations as a function of time in the microbial filter system with reeds.

Organic	Concentration, μg/ℓ						Removal, %
	Initial	(σ)	4 hr	(σ)	24 hr	(σ)	
Aromatic:							
Benzene	721.4	(223.6)	194.0	(88.0)	138.7	(73.3)	81
Biphenyl	821.0	(289.2)	38.3	(28.8)	40.5	(29.1)	95
Chlorobenzene	531.2	(191.1)	135.4	(68.0)	100.4	(76.9)	81
Dimethylphthalate	1032.6	(225.5)	321.5	(98.3)	193.9	(75.3)	81
Ethylbenzene	430.3	(196.5)	73.8	(53.1)	52.5	(46.3)	88
Naphthalene	706.7	(197.2)	69.3	(27.3)	68.0	(32.7)	90
p-Nitrotoluene	980.5	(316.3)	9.1	(18.4)	1.2	(3.0)	99
Toluene	581.4	(210.7)	103.1	(53.5)	68.9	(52.2)	88
p-Xylene	398.1	(186.2)	122.3	(107.1)	70.2	(59.9)	82

Table 3 (cont'd)

Organic	Concentration, μg/ℓ Initial	(σ)	4 hr	(σ)	24 hr	(σ)	Removal, %
Aliphatic:							
Bromoform	640.8	(246.4)	129.1	(92.1)	46.6	(52.2)	93
Chloroform	837.7	(201.3)	387.5	(103.7)	263.2	(169.8)	80
1,2-Dichloroethane	821.6	(166.3)	513.0	(116.6)	420.7	(159.7)	49
Tetrachloroethylene	457.3	(189.1)	161.7	(68.9)	112.4	(80.9)	75
1,1,1,-Trichloroethane	756.1	(291.9)	342.4	(118.2)	243.6	(119.1)	68

σ = standard deviation

Table 4. Upper limit (U) concentrations (P=0.95) for the organics after 24 hour exposure periods.

Organic	U, $\mu g/\ell$ @ 24 hr. (P=0.95)		
	Without reed	With reed	Additional reduction with reeds,%
AROMATICS:			
Benzene	425.3	261.8	38
Biphenyl	127.6	90.2	29
Chlorobenzene	357.3	229.6	36
Dimethylphthalate	560.6	320.4	43
Ethylbenzene	261.2	130.3	50
Naphthalene	149.1	122.9	18
p-Nitrotoluene	19.0	8.0	58
Toluene	219.0	156.6	28
p-Xylene	224.5	170.8	24
ALIPATHICS:			
Bromoform	347.7	134.3	61
Chloroform	632.0	548.5	13
1,2-Dichloroethane	789.9	689.0	13
Tetrachloroethylene	369.4	248.3	33
1,1,1,-Trichloroethane	561.9	443.7	21

In conclusion, this study has demonstrated the potential of a combination of reeds and biofilms to biodegrade hazardous organic compounds from natural fresh water bodies. A properly acclimated system can biodegrade both aromatic derivatives and halogenated aliphatic compounds at the same time. On an average, the systems each received 9,717 $\mu g/\ell$ of mixed chemicals spiked in river water. The reed/microbial filter reduced the mixed chemical concentration overall by 81% in 24 hrs, and the plant-free microbial filter reduced it by 70% in the same period.

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LITERATURE CITED

1. American Public Health Association. 1981. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

2. Bouwer, E. J. and P. L. McCarty. 1981. Biofilm degradation of trace chlorinated organics. Proceedings of the 1981 National Conference on Environmental Engineering. 196-202.

3. Bouwer, E. J. and P. L. McCarty. 1932a. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl. Environ. Microbiol.* 45(4):1286-1294.

4. Bouwer, E. J. and P. L. McCarty. 1983b. Transformations of halogenated organic compounds under denitrification conditions. *Appl. Environ. Microbiol.* 45(4):1295-1299.

5. Bouwer, E. J., B. E. Rittmann, and P. L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. *Environ. Sci. Technol.* 15(5):596-599.

6. Eaton, R. W. and D. W. Ribbons. 1982. Metabolism of dimethylphthalate by *Micrococcus* sp. strain 12 B. *J. Bacteriol.* 151:465-467.

7. Gibson, D. T. 1971. The microbial oxidation of aromatic hydrocarbons. *Crit. Rev. Microbiol.* 1:119-223.

8. Gibson, D. T. 1977. Biodegradation of aromatic petroleum hydrocarbons. In: D. A. Wolfe (ed.). *Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms.* Pergamon Press, New York. 39-46.

9. Government Institutes, Inc., 1985. *Environmental Statutes.* 1985 Edition. Government Institutes, Inc., Rockville, MD. 366.

10. Haber, C. L., L. N. Allen, S. Zhao, and R. S. Hanson. 1983. Methylotrophic bacteria: biochemical diversity and genetics. *Science.* 221:1147-1153.

11. Kellogg, S. T., D. K. Chatterjee, and A. M. Chakraberty. 1981. Plasmid assisted molecular breeding: new techniques for enhanced biodegradation of persistent toxic chemicals. *Science.* 214:1133-1135.

12. Keyser, P., B. G. Pujar, R. W. Eaton, and D. W. Ribbons. 1976. Biodegradation of phthalates and their esters by bacteria. *Environ. Health Perspect.* 18:159-166.

13. Longbottom, J. E. and J. J. Lichtenberg, [eds.]. 1982. *Methods for organic chemical analysis of municipal and industrial wastewater.* EPA-600/4-82-057. U. S. Environmental Protection Agency, Cincinnati, OH.

14. Ostle, B. 1972. *Statistics in Research.* The Iowa State University Press, Ames, Iowa. 89-92.

15. Rittman, B. E. and P. L. McCarty. 1980. Model of steady-state biofilm kinetics. *Biotech. and Bioeng.* 22:2343-2357.

16. Tabak, H. H., S. A. Quave, C. I. Mashni, and E. F. Barth. 1981. Biodegradability studies with organic priority pollutant compounds. *J. Water Poll. Control Fed.* 53(10):1503-1518.

17. Wilson, J. T. and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. *Appl. Environ. Microbiol.* 49(1):242-243.

18. Wolverton, B. C. 1982. Hybrid wastewater treatment system using anaerobic microorganisms and reed (*Phragmites communis*). *Econ. Bot.* 36:373-380.

19. Wolverton, B. C. and R. C. McDonald. 1981. Natural processes for treatment of organic chemical waste. *The Environ. Prof.* 3:99-104.

20. Wolverton, B. C., R. C. McDonald, and W. R. Duffer. 1983. Microorganisms and higher plants for wastewater treatment. *J. Environ. Qual.* 12(2):236-242.

21. Young, J. C. and P. L. McCarty. 1969. The anaerobic filter for waste treatment. *J. Water Pollut. Control Fed.* 41:160-173.